A STUDY OF THE MECHANISM OF INTESTINAL ABSORPTION OF BENZO(a)PYRENE

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SUMMARY

1. Benzo(a)pyrene and other polynuclear aromatic hydrocarbons are readily absorbed from the gut when fed to rats, but little is known about the mechanism of the absorption. To study the mechanism, everted sacs of small intestine were incubated in a medium containing a lipid emulsion of benzo(a)pyrene (50–1500 μM). After incubation, the concentration of benzo(a)pyrene in the sac tissue and in the medium inside the sac was determined by spectrofluorimetric analysis of chloroform extracts.

2. For incubation medium concentrations above 200 μM, accumulation of benzo(a)pyrene in the sac tissue increased exponentially with increase in incubation medium concentration. The transfer of benzo(a)pyrene from sac tissue to the inside medium was proportional to the concentration in the sac tissue. The regression lines were not greatly influenced by fasting, rat age, or sex. Comparison of incubation at 0, 23 and 42° with that at 37° showed a lowered sac accumulation at 0° and an increase in accumulation with temperature. Anaerobiosis and/or 0.1 M iodoacetate did not appreciably influence sac tissue accumulation. Benzo(a)pyrene also accumulated in strips of small and large intestine and slices of liver and kidney and epididymal fat. The feature of exponential accumulation was not limited to tissue, for adsorption isotherms and time curves for string incubated in the same media has the same forms as those obtained for the sacs.

Intragastric doses of benzo(a)pyrene appeared quickly in thoracic duct lymph and reached a peak in 3–4 h, but no more than 20% of the dose was recovered in the lymph. An exponential relationship between size of intragastric dose and concentration in adipose and mammary tissue, 18 h later, was noted.

3. The data are in accord with a mechanism of physical adsorption of benzo(a)pyrene to the intestinal mucosal surface and then passive diffusion into and through the intestinal wall. Two phases of adsorption are postulated: first, unilayer (Langmuir) adsorption, then multilayer (Polanyi) adsorption which would account for the exponential nature of the accumulation.

INTRODUCTION

There is considerable evidence, much of it long-standing, that the polynuclear aromatic hydrocarbons are absorbed through the gastrointestinal tract of the rat.

In 1940, PEACOCK[1] fed benzo(a)pyrene and then demonstrated the fluorescent material in the tissues. In an ingenious approach, SHAY *et al.*[2] placed 3-methylcholanthrene in the stomach of a lactating rat and showed the presence of 3-methylcholanthrene in the milk. Moreover, the ready induction of carcinomas in a remote organ, the mammary gland, following intragastric instillation of 3-methylcholanthrene or 7,12-dimethylbenz(a)anthracene has been demonstrated by SHAY *et al.*[3] and by HUGGINS *et al.*[4].

Though rather extensive studies have been carried out by KOTIN *et al.*[5], FLESHER[6], FLESHER AND SYDNOR[7], DAO *et al.*[8] and BOCK AND DAO[9] on the tissue concentration, distribution and metabolism of several polynuclear aromatic hydrocarbons, little work has been done on the mechanism of the intestinal absorption[10,11]. This is rather surprising when one considers the large number of studies on intestinal absorption of sugars, amino acids, fatty acids and triglycerides. No doubt the very limited water solubility of the polynuclear aromatic hydrocarbons has contributed to the lack of work in this area. In the present study, the mechanism of absorption of benzo(a)pyrene was investigated *in vitro* using the everted sac technique of WILSON AND WISEMAN[12], and *in vivo* by cannulating the thoracic ducts of rats and by determining tissue concentrations after intragastric doses of benzo(a)pyrene.

METHODS

Analysis of benzo(a)pyrene

Both tissue and medium were processed for analysis by adding 1.0 ml of medium or about 1 g of tissue to 10 ml 0.05 M H_2SO_4. After homogenization with a VirTis 40 blender, the polycyclic hydrocarbon was extracted into 15 ml of chloroform. After dilution of the chloroform extract to an extent sufficient to avoid significant quenching (internal standards were performed on individual samples to determine whether quenching was present) excitation was carried out at 300 nm and fluorescence measured at 425 nm. In experiments in which tritiated benzo(a)pyrene was utilized, the concentration was determined both by spectrofluorimetry and by liquid scintillation counting. In experiments to detect metabolic alterations in the benzo(a)pyrene molecule, an aliquot of chloroform extract was evaporated to dryness under N_2 and then redissolved in a small amount of fresh chloroform. Thin-layer chromatography was carried out with Baker-Flex^R silica gel IB, and benzene–absolute ethanol (95:5, v/v) was utilized as solvent. The excitation and fluorescent spectra of chloroform extracts of media and tissue after incubation were also obtained in many experiments.

Everted sac technique

The small intestine of the rat was removed, the lumen rinsed with 0.9% (w/v)NaCl, and four segments of approximately equal length were cut. Each quartered segment was everted (so that the mucosa was exterior) and divided in half (to give two sacs per quarter). Each sac was tied at one end, and after filling the sac with (serosal) medium the other end was tied. The inside (serosal) solution was the same as that in the external (mucosal) incubation medium except that it did not contain the polynuclear aromatic hydrocarbon. Two sacs were placed in each 50-ml flask containing 15 ml of medium; thus each flask represented one of the four quarters

of the intestine. Unless specified otherwise, incubations were carried out for 1 h at 37° in a Dubnoff water bath shaker (100 cycles/min); O_2–CO_2 (95:5, by vol.) or N_2–CO_2 (95:5, by vol.) was continually bubbled through each flask. The sacs were weighed empty, and again after filling with inside medium and at the end of the experiment. Sprague–Dawley rats were used. Strings were removed from the sacs before the sac tissue was prepared for analysis.

Incubation medium

Tissue culture medium (Eagle's minimal essential medium)[13] containing 20 % hypogamma calf serum served as the basic incubation medium. The electrolyte concentration of this medium corresponds to that of extracellular fluid, but vitamins and amino acids are also present. Benzo(a)pyrene was added in the following manner: 1 part Tween 20, 1 part lecithin and 10 parts sesame oil containing the benzo(a)-pyrene, were emulsified at 40000 rev./min with a VirTis blender. 1 part of this emulsion was added to 99 parts of the basic incubation medium and mixed again with the VirTis blender. The medium was filtered through several layers of cheese cloth to remove any flakes of unemulsified benzo(a)pyrene. Emulsion droplets were sized with a Coulter counter plotter and by microscopic examination with a calibrated grid. Most droplets were much less than 1 μ in diameter. The size distribution pattern could not be distinguished from the pattern of chylomicrons in rat thoracic duct lymph.

Tissue strips and slices

The stomach and intestines were incised longitudinally and then cut into 1-cm lengths. Liver and kidney were sliced at a thickness of about 0.5 mm by techniques utilized for tissue respiration studies[14]. Epididymal fat pads were also studied. These incubations were carried out under aerobic conditions as described above.

In vivo experiments

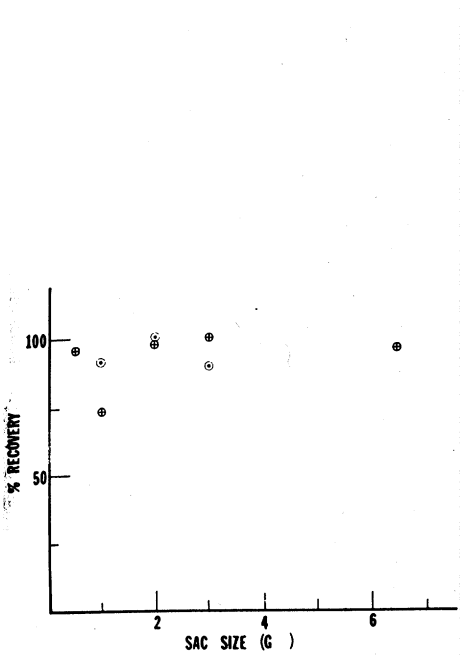
Benzo(a)pyrene was dissolved in 1 ml sesame oil and introduced intragastrically with soft rubber catheter into male rats of the Sprague–Dawley strain. The dose of benzo(a)pyrene varied with the experiment. The concentration of benzo(a)-pyrene in breast and in adipose tissue of the rat was determined 18 h after the dose was given. In some experiments the lymphatic duct was cannulated *via* the Bollman technique as modified by GALLO-TORRES AND MILLER[15] and 0.9 % (w/v) NaCl was introduced *via* a duodenal cannula at a rate of 3 ml/h with a peristaltic pump in order to assure lymph flow. Lymph was collected over timed intervals and extracted into chloroform as indicated in the procedure above.

RESULTS

Completeness of benzo(a)pyrene extraction

In order to determine the completeness of extraction of benzo(a)pyrene from tissue, 600–8600 μg of benzo(a)pyrene dissolved in 0.1 ml chloroform was applied directly to quarters of everted rat small intestine. The benzo(a)pyrene was permitted to soak into the tissue for 30 min after which it was extracted by the usual procedure. This was carried out on twelve different quarters of intestine from three different

rats. The mean recovery was 94 ± 13 % (± S.D.). The results of an experiment (Fig. 1), using sacs of different sizes and different amounts of benzo(a)pyrene, indicated that the amount of tissue and the amount of benzo(a)pyrene present did not influence the recovery.



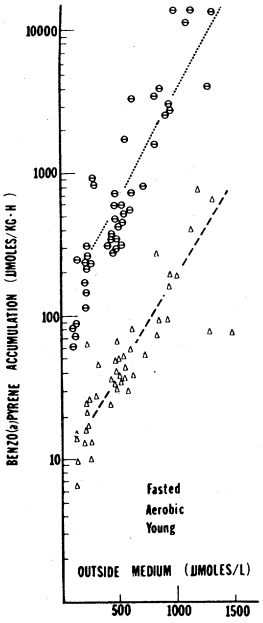


Fig. 1. Recovery of benzo(a)pyrene extracted 30 min after its direct application to small intestinal sacs of varying size. Benzo(a)pyrene added: ⊙, 5000 μg; ⊕, 833 μg.

Fig. 2. Accumulation of benzo(a)pyrene in sac tissue (upper regression line) and inside medium (lower regression line) after 1 h incubation in a O_2–CO_2 (95:5, by vol.) atmosphere. Tissue obtained from 40–60-days-old rats which had been fasted 16–18 h prior to sacrifice.

Accumulation of benzo(a)pyrene in everted sacs.

The pattern of accumulation of benzo(a)pyrene in the intestinal sacs was unusual (Fig. 2). When the outside incubation medium had a benzo(a)pyrene concentration above 200 μM, the accumulation of benzo(a)pyrene in the sac tissue increased exponentially with increase in concentration of benzo(a)pyrene in the incubation medium. At concentrations above 1500 μM, there was no further increase in sac tissue concentration; in fact, in some experiments the sac tissue content was lower than that observed at 1500 μM. (It was difficult to prepare incubation medium with concentrations above 1500 μM, and on some occasions when concentrations greater than this were obtained, yellow crystals of benzo(a)pyrene were visible on the sacs and in the strips.) No difference in accumulation in the four segments of intestine tissue was noted. When the mucosal layer was removed from the underlying muscularis, analysis of extracts from these two layers showed that the mucosa had the higher concentration.

The amount of benzo(a)pyrene transported through sac tissue to the medium inside the sacs was directly proportional to the sac tissue accumulation. This is

indicated by the parallel relationship between the regression lines. In these experiments the medium inside the sacs was removed by making a small cut with scissors in the wall at the extreme lower end of sacs held vertically above a collecting beaker. This procedure introduces some benzo(a)pyrene from the mucosa into the collected inside media, since (in separate experiments) media removed carefully with a needle and syringe were found to have a benzo(a)pyrene concentration 50–60 % that of media removed from companion sacs by the cutting technique used in these experiments.

Effects of rat age and fasting on sac accumulation

Rats 40–60 days old were compared with 80–100-days-old rats both in the fasted and non-fasted state. Fasting was started 16–18 h prior to an experiment but water was available *ad libitum*. The slopes and intercepts of the regression lines relating sac tissue accumulation and incubation medium concentration are given in Table I. The intercept for non-fasted animals was greater than that obtained

TABLE I

SLOPES AND INTERCEPTS OF THE REGRESSION LINES RELATING SAC TISSUE ACCUMULATION AND INCUBATION MEDIUM CONCENTRATION

Age		*Aerobic Sacs*		*Anaerobic Sac*
		Fasted	*Non fasted*	*Fasted*
40–60 days	Intercept*	103	407	105
	Slope	$1.6 \cdot 10^{-3}$	$0.71 \cdot 10^{-3}$	$0.91 \cdot 10^{-3}$
80–100 days	Intercept*	230	369	
	Slope	$1.0 \cdot 10^{-3}$	$0.49 \cdot 10^{-3}$	

* μmoles/kg per h.

for fasted animals, but the slope was greater for fasted animals. In the non-fasted animals the scatter of data was greater than that obtained for fasted animals. This difference may have been due to differences in extent of feeding (before the experiment), a factor which was not experimentally controlled in the non-fasting animals. The intercept for the older fasted rats was greater than that for the younger fasted animals. The slope was somewhat less for the latter. Age made less difference in the case of the non-fasted animal. Although most of the data presented were obtained from male rats, female rats were also studied but no difference due to sex was detected.

Reversibility of adsorption on the mucosal surface

The reversibility of benzo(a)pyrene adsorption was tested in the following way. The entire small intestine was used in preparing nine everted sacs which were distributed randomly into three separate flasks, three sacs per flask. The sacs were incubated in medium containing benzo(a)pyrene (576 μM). At the end of 1 h, one sac from each flask was removed for analysis (Group A) and the result obtained provided a baseline for benzo(a)pyrene adsorption. A second sac from each flask was transferred to fresh medium without benzo(a)pyrene and incubated for a second

hour (Group B), and the third sac in each flask was incubated for a second hour in the original benzo(a)pyrene-containing medium (Group C). The sac tissue concentrations of benzo(a)pyrene averaged 510 μmoles/kg in Group A; the further incubation gave values of 270 μmoles/kg for sacs in Group B and 945 μmoles/kg for the sacs in Group C. The reversibility of benzo(a)pyrene adsorption is indicated by desorption to the extent of 47 % in the case of sacs incubated in medium without benzo(a)pyrene. In contrast, the adsorption of benzo(a)pyrene onto sac tissue increased during the second hour of incubation in medium containing benzo(a)pyrene.

Noneverted versus everted sacs

Five experiments were directed towards comparing everted (mucosal surface outside) with noneverted (serosal surface outside) sacs. In each experiment, sacs from the first and third quarter of the intestine were everted while sacs from the second and fourth were not. The sac tissue accumulation through the mucosal (everted surface) was on the average of 3.0 $\pm$ 0.2 ($\pm$ S.E.) times greater than that through the serosal (noneverted) surface.

Effect of temperature

Although most experiments involved incubation at 37°, a few were carried out at 0°, 25°, and 42°, in order to detect any effect of temperature on sac tissue accumulation. Sacs were incubated in the same medium at 37° and at the other temperatures. Results (Table II) are expressed relative to the accumulation at 37°.

TABLE II

EFFECT OF TEMPERATURE ON ACCUMULATION OF BENZO(A)PYRENE IN SAC TISSUE

	0°/37°	*23°/37°*	*42°/37°*
Mean $\pm$ S.E.	0.4 $\pm$ 0.06	0.9 $\pm$ 0.06	1.11 $\pm$ 0.09
Number of comparisons	6	8	8
Range of concentration* (μM)	510–635	400–475	600–735

* There was no systematic variation of the ratio with concentration of incubation medium.

Effect of anaerobiosis and iodoacetate

Anaerobiosis affected the accumulation of benzo(a)pyrene in the sac tissue only to the extent of changing the slope of the regression line somewhat (Fig. 3 and Table I). Iodoacetate (0.1 M), which inhibits glycolysis, had no obvious effect on sac tissue accumulation whether the sacs were incubated under aerobic or anaerobic conditions. In these experiments two sacs were incubated in medium with iodoacetate and two sacs in medium without this inhibitor. Under aerobic conditions, the benzo(a)pyrene concentration in the presence of iodoacetate was 1.00 $\pm$ 0.14 times that of the control sacs; anaerobically (also in the presence of iodoacetate) the result was 1.15 $\pm$ 0.18. Thus, the accumulation of benzo(a)pyrene in the everted sac does not seem dependent on the generation of metabolic energy.

The accumulation of benzo(a)pyrene with time

The accumulation of benzo(a)pyrene in sacs removed after different intervals of incubation was determined (Fig. 4). The first sacs were taken 0.5 min after being

placed in incubation medium. There was an initial, very rapid uptake of benzo(a)-pyrene. The data also suggest that, at higher concentrations of benzo(a)pyrene in the incubation media, equilibrium (between the sac tissue concentration and the incubation medium concentration) was not completely attained in 1 h.

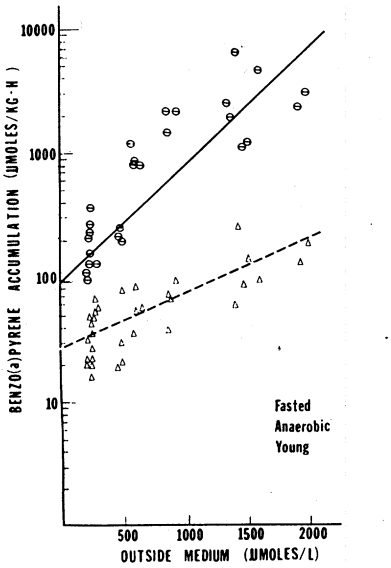


Fig. 3. Accumulation of benzo(a)pyrene in sac tissue (upper regression line) and inside medium (lower regression line) after incubation for 1 h in a N_2–CO_2(95:5, by vol.) atmosphere. Tissue obtained from 40–60-days-old rats which had been fasted for 16–18 h prior to sacrifice.

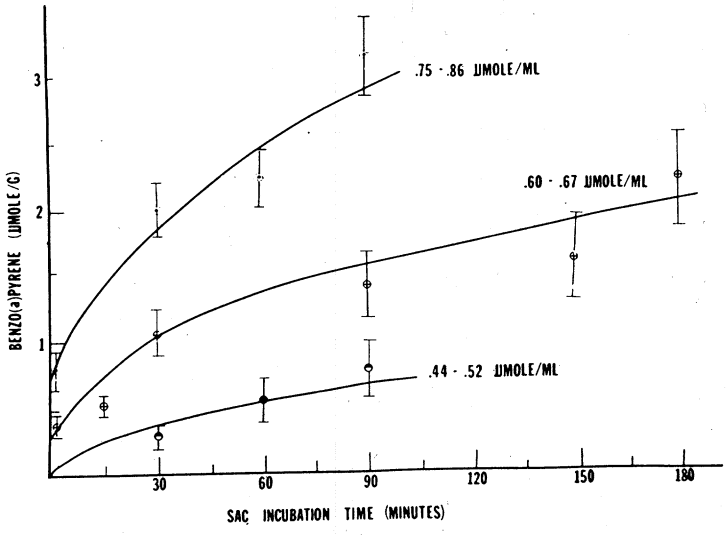


Fig. 4. Relationship between accumulation of benzo(a)pyrene in sac tissue and the duration of incubation in a O_2–CO_2(95:5, by vol.) atmosphere. Experiments were performed at three levels of medium concentration: a, 0.75–0.86 μmole/ml; b, 0.60–0.67 μmole/ml; c, 0.44–0.52 μmole/ml. Tissue obtained from 40–60-days-old rats which had been fasted 16–18 h prior to sacrifice.

Thin-layer chromotography

In the system used, benzo(a)pyrene had an R_F value of 0.70–0.75. No other fluorescent spots were seen in extracts of sac tissue or incubation media; however, a second less intense fluorescent spot was seen in extracts of inside media. The R_F value of this spot was 0.45–0.55, but the compound has not been further characterized.

Accumulation of benzo(a)pyrene in tissue strips and slices

Benzo(a)pyrene accumulates not only in the tissue from the small intestine but also in slices prepared from other tissue (Table III). The accumulation in the strips of small intestine was not as great as that observed in the sac experiments, possibly because the mucosa of strips is more compact (*i.e.* the intestinal wall is not under a strectching tension as it is in the case of sacs).

TABLE III

BENZO(a)PYRENE CONCENTRATION IN TISSUE STRIPS AND SLICES AFTER 1-h INCUBATION

	*Expt. 1**	*Expt. 2***
	Tissue concentration (μmoles/kg)	
Stomach	357	254
Small intestine, 1st	382	377
Small intestine, 2nd	238	178
Small intestine, 3rd	405	237
Small intestine, 4th	321	285
Colon, 1st (cecum)	281	480
Colon, 2nd	354	293
Colon, 3rd	277	201
Liver slices	710	217
Kidney slices	246	226
Epididymal fat pad	520	173

* Initial medium concentration 600 μmoles/l.
** Initial medium concentration 300 μmoles/l.

Adsorption onto string

Since physical adsorption of benzo(a)pyrene on the intestinal surface seemed to be involved in the initial step of absorption, the adsorption on an inanimate surface was studied. Four strings of suture material, 7 cm in length and 4 mg in weight, were shaken in the incubation medium for 1 h after which the medium and the strings were extracted in the same manner as before. An exponential relation (Fig. 5) was obtained. In a later series of experiments, the adsorption of benzo(a)-pyrene onto string was studied as a function of time (Fig. 6); in these experiments ordinary white string, 7 cm in length and 28–30 mg in weight was used. Incubation was carried out in the same manner as the intestinal sacs, including the bubbling of O_2–CO_2(95:5, by vol.) through 15 ml of medium. Results were comparable to those obtained with intestinal sacs.

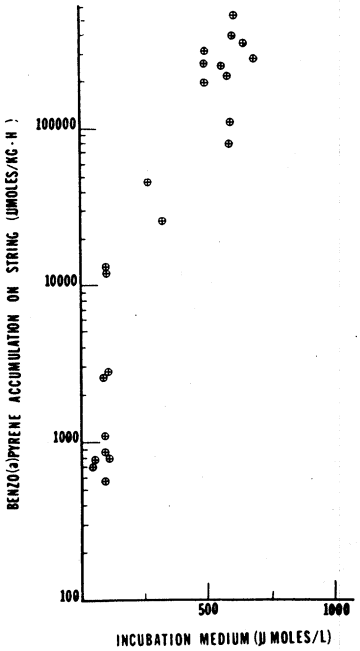
Appearance of benzo(a)pyrene in the lymph

10 mg of benzo(a)pyrene was given intragastrically in 1 ml of sesame oil. The compound quickly appeared in the thoracic duct lymph, reached a peak during

the third and fourth hour and then decreased rather rapidly (Fig. 7). Only 10–20 % of the administered dose appeared in the lymph of these cannulated animals.

In vivo accumulation in adipose tissues

Various doses of benzo(a)pyrene dissolved in 1 ml of sesame oil were fed (by gastric tube) to 50–60-days-old female rats, 18 h later the animals were dec-



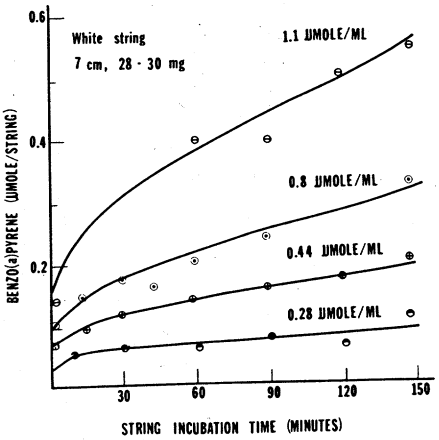


Fig. 5. Relationship between accumulation of benzo(a)pyrene on black suture string and medium concentration. 1-h incubation in a O_2–CO_2(95:5, by vol.) atmosphere.

Fig. 6. Relationship between accumulation of benzo(a)pyrene on white, 1-mm-diameter string and duration of incubation. O_2–CO_2 (95:5, by vol.) atmosphere. Experiments were performed at three levels of medium concentration.

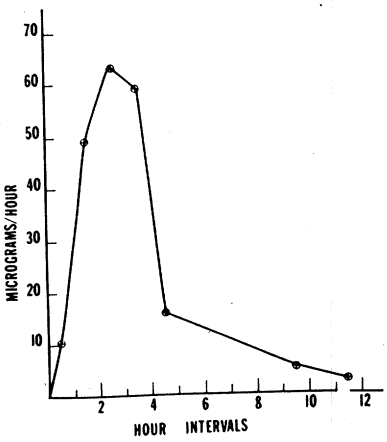


Fig. 7. Time-course of appearance of benzo(a)pyrene in thoracic duct lymph following intragastric administration.

pitated and the concentration of the compound in the retroperitoneal fat and mammary tissue determined. The tissue concentration increased exponentially with dose in both tissues (Fig. 8).

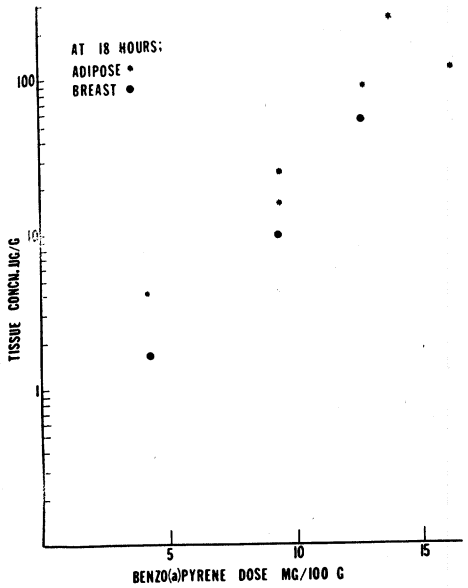


Fig. 8. Relationship between benzo(a)pyrene accumulation in mammary and retroperitoneal adipose tissue and dose administered intragastrically 18 h prior to sacrifice. 50–60-days-old female rats were utilized.

DISCUSSION

Though the intestinal absorption of sugars and amino acids seems to follow Michaelis–Menten kinetics, it is evident from our experiments that benzo(a)pyrene absorption (at concentrations above about 200 μM) does not. Any proposed mechanism for absorption must account for (a) the exponential increase in sac tissue concentration as the concentration of benzo(a)pyrene increases in the incubation medium and (b) the intercept on the y-axis, which is high above the value of 2–3 μmoles/kg per h equivalent in background fluorescence associated with the tissue or with the incubation medium. Since anaerobiosis and inhibition of glycolysis with iodoacetate (both of which deprive cells of energy supplies) do not greatly alter the regression line relating sac tissue accumulation to incubation medium concentration, our attention was directed toward a process of physical adsorption. The appropriateness of this notion is strengthened by the formal similarity between intestinal sacs and lengths of string with respect to their adsorption isotherms and kinetic curves.

Multilayer adsorption, the theory and status of which have been reviewed by ADAMSON[16,17] and by HANSEN AND SMOLDERS[18] can account for the exponential part of the sac tissue accumulation line in Fig. 2. By using this concept of physical adsorption the first step in the intestinal absorption of benzo(a)pyrene can be

pictured as follows: Molecules of benzo(a)pyrene adsorb onto surface sites of the intestinal mucosa according to the Langmuir isotherm. Saturation of the sites can be considered to represent completion of a unilayer. The *y*-intercept (*i.e.* sac tissue concentration regression line extrapolated to zero concentration of benzo(a)-pyrene in the incubation medium) can be interpreted to approximate the amount of benzo(a)pyrene required to saturate the sites present on a 1-g sac of everted intestine. Increases in medium concentration above 200 μM lead to multilayer adsorption according to the now accepted POLANYI potential theory[16,19]. This is an exponential phenomenon. Although most studies of physical adsorption have been concerned with the adsorption of gases on solids, work has also been done with liquid solutions. It is germane that multilayer adsorption of aliphatic alcohols and acids from aqueous solutions onto carbon particles has been shown to occur[20,21].

The second step in the process of intestinal absorption involves passive diffusion of benzo(a)pyrene from the adsorbed layer on the mucosa through the intestinal wall into the medium within the everted sac. The parallel regression lines for sac tissue accumulation and for transport into the inside medium are consistent with a passive diffusion process. Thus, the mechanism of the intestinal absorption of benzo(a)pyrene seems to involve the processes of physical adsorption and passive diffusion.

Our findings are not unique to benzo(a)pyrene; data[11] obtained for other polycyclic hydrocarbons (*e.g.* 3-methylcholanthrene, 7,12-dimethylbenz(a)anthracene, chrysene, and 1,2-benzanthracene) are comparable, though with some differences in slopes and intercepts. In addition, there are similarities between the intestinal transport of benzo(a)pyrene and the transport of lipids in everted sacs. JOHNSTON AND BORGSTROM[22] noted that micellar solutions of fatty acids accumulated in slices of boiled hamster intestine; metabolic inhibitors also had little effect on fatty acid uptake although the resynthesis of triglycerides was affected. Likewise, FELDMAN[23] found that inhibition of energy-yielding reactions in rings of hamster intestine had only a small influence on cholesterol uptake. In a later study by FELDMAN[24] kinetic and temperature coefficient experiments suggested an adsorption or diffusion process. However, the exponential increase observed for benzo(a)pyrene and other polycyclic hydrocarbons is not evident in the data plotted for fatty acid transport by JOHNSTON AND BORGSTROM[22] and for cholesterol by SMITH AND TREADWELL[25] and FELDMAN[24]. The very high lipophilicity and hydrophobicity of benzo(a)pyrene along with the planar aromatic structure of the molecule may account for this difference.

From the physical-chemical and physiologic viewpoints, the conditions of our experiments are quite complicated. The mucosal surface of the intestinal sac is not flat but is covered with convolutions (villi), which in turn contain a large number of projecting filaments (microvilli). In addition, the benzo(a)pyrene is not in aqueous solution but is dissolved in a lipid emulsion containing several other components. Moreover, lipid absorption probably accompanies benzo(a)pyrene absorption. The relationship of these two processes remains to be studied in detail.

With respect to carcinogenesis, two implications of the exponential relationship between sac tissue accumulation and concentration of benzo(a)pyrene in the incubation medium should be considered. First, the exponential relationship implies that as the concentration of polycyclic hydrocarbon to which the intestinal mucosa

is exposed is increased, the rate of absorption accelerates. For example, if a certain increase in concentration of benzo(a)pyrene in the medium results in a 10-fold increase in absorption, then a second linear increase of the same amount would increase absorption 100-fold (rather than 20-fold). This stresses, for reasons not previously recognized, the importance of maintaining low concentrations of such substances in the environment to which man and his tissues are exposed. Secondly, if the ability to absorb benzo(a)pyrene from the extracellular fluid (including blood) to the cells in the various tissues of the body also has an exponential relationship, then the rate of absorption of polycylcic hydrocarbons from the intestine becomes very important. If the rate of absorption is rapid, then the level in the extracellular fluid would become high and the amount deposited in the cells of various tissues would be greater according to the exponential relationship. Although we have not performed *in vivo* experiments to determine the relationship between peak blood levels and dose of benzo(a)pyrene, our data do indicate an exponential relationship between dose and subsequent concentration of benzo(a)pyrene in the adipose tissues.

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